

CLAIMS:

1. A composition for growing pluripotent cells and/or for directing their differentiation, said composition including a factor or factors capable of inhibiting an activity of bone morphogenetic protein-2 (BMP-2).
- 5 2. A composition according to claim 1 wherein said pluripotent cells are embryonic stem (ES) or embryonic germ (EG) cells.
3. A composition according to claim 2 wherein said ES or EG cells are human ES or EG cells.
4. A composition according to claim 1 wherein said factor is derived from a
10 primitive endoderm cell line.
5. A composition according to claim 4 wherein said factor is selected from the group consisting of a soluble factor or membrane bound factor shed by said cell line or an extra-cellular matrix component produced by said cell line.
6. A composition according to claim 5 wherein said primitive endoderm cell
15 line is derived from a testicular teratocarcinoma.
7. A composition according to claim 6 wherein said cell line is primitive endoderm cell line GCT 44, as hereinbefore described.
8. A composition according to claim 1 wherein said factor is selected from the
20 group consisting of chordin, noggin, DAN, cerebrus, a modified BMP-2 receptor which is capable of binding BMP-2 but does not activate signal transduction pathways associated with BMP-2 induced differentiation, and small molecules which interfere with signal transduction pathways involved in BMP-2 induction of pluripotent cell differentiation.
9. A composition according to claim 1 wherein said factor is present in said
25 composition in a concentration capable of inhibiting extraembryonic

differentiation of EC or ES cells.

10. A composition according to claim 1 further including a secondary factor, said secondary factor including a ligand or ligands.
- 5 11. A composition according to claim 10 wherein said ligand is selected from the group consisting of CD30 and functionally equivalent molecules and ligands of the Notch family of receptors.
12. A method of regulating growth and/or differentiation of pluripotent cells, said method including culturing said cells in the presence of a factor or factors capable of inhibiting an activity of BMP-2.
- 10 13. A method according to claim 12 including providing
 - a pluripotent cell line, and
 - an effective amount of said factor; andculturing the cell line in the culture medium.
- 15 14. A method according to claim 13 wherein said pluripotent cells are embryonic stem (ES) or embryonic germ (EG) cells.
15. A method according to claim 14 wherein said ES or EG cells are human ES or EG cells.
16. A method according to claim 13 wherein said factor is derived from a primitive endoderm cell line.
- 20 17. A method according to claim 16 wherein said factor is selected from the group consisting of a soluble factor or membrane bound factor shed by said cell line or an extra-cellular matrix component produced by said cell line.
18. A method according to claim 17 wherein said primitive endoderm cell line is

derived from a testicular teratocarcinoma.

19. A method according to claim 18 wherein said cell line is primitive endoderm cell line GCT 44, as hereinbefore described.
- 5 20. A method according to claim 13 wherein said factor is selected from the group consisting of chordin, noggin, DAN, cerebrus, a modified BMP-2 receptor which is capable of binding BMP-2 but does not activate signal transduction pathways associated with BMP-2 induced differentiation, and small molecules which interfere with signal transduction pathways involved in BMP-2 induction of pluripotential cell differentiation.
- 10 21. A method according to claim 13 wherein said factor is present in the culture medium in a concentration capable of inhibiting extraembryonic differentiation of EC or ES cells.
22. A method according to claim 13 further including a secondary factor, said secondary factor including a ligand or ligands.
- 15 23. A method according to claim 22 wherein said ligand is selected from the group consisting of CD30 and functionally equivalent molecules and ligands of the Notch family of receptors.
24. A method for producing a factor or group of factors capable of antagonising an action of BMP-2 on pluripotential cells, said method including
20 providing
a primitive endoderm cell line, and
a suitable culture medium; and
culturing the cell line in the culture medium for a period of time sufficient to produce said factor.
- 25 25. A method according to claim 24 wherein said factor is selected from the group consisting of a soluble factor or membrane bound factor shed by said

cell line or an extra-cellular matrix component produced by said cell line.

26. A method according to claim 25 wherein said primitive endoderm cell line is derived from a testicular teratocarcinoma.
27. A method according to claim 26 wherein said cell line is primitive endoderm cell line GCT 44, as hereinbefore described.
28. A method according to claim 24 wherein said suitable culture medium is a serum-free medium.
29. A method according to claim 28 wherein said medium is IMDM.
30. A method according to claim 24 wherein said period of time is approximately 3 to 4 weeks.
31. A method according to claim 24 further including subjecting the factor so produced to a purification step.
32. A method according to claim 31 wherein said purification step is selected from the group consisting of tangential flow filtration, anionic exchange, cationic exchange, reverse phase chromatography and combinations thereof.
33. A method according to claim 31 further including assaying for the factor so produced.
34. A method according to claim 33 wherein said assaying is performed during said purification step.
35. A method according to claim 33 wherein said assaying is performed using a bioassay using GCT type multipotent cells grown in the presence of BMP-2.
36. A pluripotential cell or cell line or a differentiated cell or cell line produced using the composition of claim 1.

37. A pluripotential cell or cell line or a differentiated cell or cell line produced by the method of claim 12.
 38. An embryo or transgenic embryo derived from the cells of claim 36 or 37.
 39. An animal or transgenic animal produced from the embryo or transgenic embryo of claim 38.
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